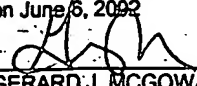


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Signature

**PATENT**  
#01-0367-UNI  
Case #F7590(V)

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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant: Van Oorschot et al.

Serial No.: 10/072,570

Filed: February 8, 2002

For: PROCESS FOR THE PREPARATION OF ONE OR MORE STATINS BY  
FERMENTATION

Edgewater, New Jersey 07020  
June 6, 2002

**SUBMISSION OF PRIORITY DOCUMENT**

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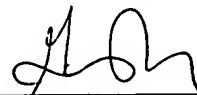
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Pursuant to rule 55(b) of the Rules of Practice in Patent Cases, Applicant(s) is/are submitting herewith a certified copy of the European Application No. 01200489.1 filed February 9, 2001, and European Application No. 01200493.3 filed February 9, 2001, upon which the claim for priority under 35 U.S.C. § 119 was made in the United States.

It is respectfully requested that the priority document be made part of the file history.

Respectfully submitted,

  
Gerard J. McGowan, Jr.  
Reg. No. 29,412  
Attorney for Applicant(s)

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**Blatt 2 der Bescheinigung**  
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**Page 2 de l'attestation**

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Titre de l'invention:

Process for the preparation of statins by fermentation using soy-bean as substrate

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

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Process for the preparation of one or more statins by  
fermentation

## 5 Description

### Technical field

The present invention relates to process for the preparation of  
10 one or more statins by fermentation. The invention further  
relates to food product comprising one or more statins.

### Background of the invention

15 Statins are compounds that are known to have a lowering effect  
on levels of low-density lipoprotein cholesterol (LDL-  
cholesterol) in the human blood. Elevated LDL-cholesterol  
levels (hypercholesterolemia) is directly related to increased  
risk of coronary heart disease. Statins inhibit the  
20 hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, the rate-  
determining step in the cholesterol biosynthesis.

Scientific research has confirmed the healthy properties of  
statins especially with respect to LDL blood-cholesterol and  
25 triglyceride levels lowering activities, both in animals and in  
humans (Li et al., Nutrition Research 18, 71-81 (1998); Heber  
et al., Am. J. Clin. Nutr. 69, 231-236 (1999)).

Early reports of the effect of statins were made in 1979. The  
30 Japanese scientist Endo isolated a metabolite from *Monascus*  
that reduced artificially induced hyperlipoproteinemia in rats  
(Endo, J. Antibiotics 32, 852-854, (1979)). These metabolites  
are known as monacolins. Monacolin is identical to the

cholesterol lowering pharmaceutical lovastatin. Lovastatin is sold by Merck co. under the tradename Mevacor. A derivative of lovastatin, simvastatin, is sold as a cholesterol-lowering drug under the name of Zocor. Other derivatives of lovastatin e.g. pravastatin, and mevastatin, are also sold as lipid lowering drugs against hypercholesterolemia. *Monascus*-extracts are sold in capsules in Japan as the dietary product Monacolin. The usual dose of the above statins is 20 mg/day, which results in at least 20% blood LDL-cholesterol lowering.

10

The production of statins is also reported in fermentation using fungi other than the above-mentioned *Monascus* species. It has been shown that statins can be produced by a variety of filamentous fungi, including *Monascus*, *Aspergillus*, *Penicillium*, *Pleurotus*, *Pythium*, *Hypomyces*, *Paecilomyces*, *Eupenicillium*, and *Doratomyces*.

15

The preparation and purification of the statins used in pharmaceutical preparations involves many process-steps, in which ingredients are used that are not commonly used in the food industry. The many process steps increase costs compared to processes having less process steps. For these reasons the statins prepared for pharmaceutical use are not used in the foods industry.

25

As a food product, rice fermented with a red *Monascus* fungus (red rice) has been known and used for hundreds of years in China. Red rice was used and still is used in wine making, as a food-colouring agent and as drug in traditional Chinese medicine. We have found that most red rice available on the market contains no statins or statins in very low amounts. The Food and Drug Administration has concluded that red yeast rice

30



available in the market does not contain significant amounts of lovastatin (FDA, Docket No. 97-0441, Final Decision).

WO 99/23996 describes a composition for treating elevated serum  
5 cholesterol and/or triglycerides comprising a red rice product containing at least 0.05% lovastatin by weight.

Red rice powder capsules are sold as dietary supplements under the name of Cholestin by the firm Pharmanex. Pharmanex also  
10 sells a Cholestin bar containing red yeast rice (*Monascus purpureus* went).

Red rice has an intensive red colour. Whereas the intensive red colour of red rice is an advantage when it is used as colouring  
15 agent, it is a disadvantage when it is used in food products. Due to the intense red colour of red-rice products, the foods prepared from red rice are coloured, depending on the amount of red-rice product added to the food product yellow, orange or red. The higher the amount of red rice added to the food, the  
20 more intense is the red colour of the food product. In the known food products a relatively large amount of red rice has to be added in order to add enough statins. This results in a red colour of the products that cannot be avoided.

25 In some food products the red rice colouring is undesirable. In particular in the western world, consumers are reluctant to use products of which the colour has changed from that they are used to. For example spreads, including margarine, butter, halvarin or salad oils are considered unacceptable by  
30 customers, when the colour of such a product is orange or red. However, at the same time these type of products have been found by us to be excellent vehicles of the daily intake of

amounts of statins sufficient to obtain a blood LDL-cholesterol lowering effect.

#### Summary of the invention

5

It is an object of the invention to provide a food product that has no undesired colouring due to the addition of statins. A further object of the invention is to increase the health effects of the known food products comprising statin. Another  
10 object is to provide a process for the preparation of a food product comprising statin, which involves less process steps than in the preparation of statins as a pharmaceutical drug. Another object is to provide such a process, which avoids the use of ingredients or process aids that are not commonly used  
15 in the food industry.

One or more of these objects are attained by a process for the preparation of one or more statins by fermentation, wherein a substrate is fermented with statins producing fungus,  
20 characterized in that substrate comprises more than 20% by weight of soy ingredients.

Further one or more of the above objects are attained by a food product comprising:

- 25 a) an amount of one or more statins  
b) an amount of one or more compounds chosen from the group: polyunsaturated fatty acids, phytosterols, proteins, peptides, dietary fibers, including soluble fibers, polyphenols and saponins, wherein the food product has a Hue a value of less  
30 than 20, preferably less than 20, most preferably less than 0.

Preferably the amount of a) is 5-100 mg/kg and the amount of b) 1 wt. or higher. More preferably the amount of b) is 5 wt.% or higher.

35

We have found that when the substrate for the *Monascus* fermentation is soybeans and/or soybean ingredients, the red colouring of the fermented product as in red rice fermentation is avoided, i.e. a non-coloured or only slightly coloured fermentation product is obtained. Further we have found that compounds having a positive health effect, which are present in soybeans are also present in the fermented product. These compounds include, but are not limited to polyunsaturated fatty acids, phytosterols, proteins, peptides, dietary fibers including soluble fibers, polyphenols and saponins. As a result of the presence of these compounds in the fermentation product, the food product according to the invention has increased health effects compared to the known food products comprising statin.

15

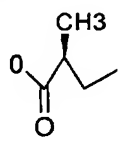
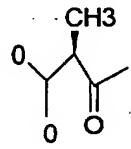
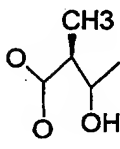
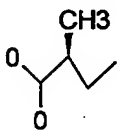
#### Detailed description of the invention

The following definitions will be used.

20 Statins are defined as substances having the structural formula, presented in figure 1. In this structural formula, R1 and R2 can be any group. Preferred statins are those which are given in table 1.

6

Table 1: Preferred statins according to formula of figure 1

	R1	R2
monakolin K	CH3	
monakolin L	CH3	H
monakolin J	CH3	OH
monakolin X	CH3	
monakolin M	CH3	
compactin (ML-236B)	H	
ML-236-A	H	OH
NL-236-C	H	H

Polyphenols herein are polyphenols having plant origin. These include flavenoids, which include isoflavones.

The polyphenols include isoflavones, stilbenes, lignans, coumestans and resorcylic acid lactones. Examples of

5 isoflavones are genistein, daidzein, equol, glycitein, biochanin A, coumestrol, maitaresinol, formononetin, O-desmethylenegalesin, enterolactone and enterodiol. Preferred isoflavones according to the invention are genistein and daidzein and glycitein, which are present in soybeans.

10

Saponins are herein derived as  $\beta$ -D-glucopyranosiduronic acid derivatives. Examples of saponins are Soya sapogenol A,B,C,D and E, Soyasaponin I, II and III, as described in Lebensmittel

15 1993, pages 550-552.

Polyunsaturated fatty acid esters are defined as fatty acid esters having more than one unsaturated group in the fatty acid chain. Examples of polyunsaturated fatty acid esters are

20 linoleic acid esters, linolenic acid esters, arachidonic acid esters.

Dietary fibers are herein a collective term for a variety of plant substances that are resistant to digestion by the human

25 gastrointestinal enzymes. Depending on their solubility, dietary fibers can be classified into insoluble (cellulose, some hemicelluloses, lignins), and soluble (remainder of the hemicelluloses, gums, mucilages. Soybean cotyledon fibers comprise both soluble and insoluble dietary fibers.

30

Phytosterols are herein defined as sterol compounds produced by plants, which are structurally very similar to cholesterol except that they contain some substitutions at the C24 position

on the sterol side chain. The phytosterols include 4-desmethysterols, 4-monomethylsterols, 4,4'-dimethylsterols and mixtures thereof. Examples of such phytosterols are  $\beta$ -sitosterol, campesterol, stigmasterol. The term phytosterols  
5 herein also includes phytostanols, the saturated equivalents of phytosterols.

Polyphenols, polyunsaturated fatty acids, phytosterols, proteins, peptides, dietary fibers, and saponins will  
10 hereinafter collectively be referred to as soy actives.

The amounts given will be expressed, in wt.% or weight parts per million (ppm), mg/kg or g/kg, relative to the total weight of the food product, unless otherwise indicated.

15

The amounts of statins given herein are the sum of the amounts of individual statins, as e.g. determined by chromatography, unless otherwise indicated.

20 The protein amounts given herein are the sum of the amounts of individual proteins, unless otherwise indicated.

The amounts of soy actives are expressed as the sum (wt.% or ppm) of polyunsaturated fatty acids, phytosterols, proteins,  
25 peptides, dietary fibers including soluble fibers, polyphenols and saponins.

The food product has a Hue a\* value of less than 20, preferably less than 20, most preferably less than 0. The Hue a\* value is  
30 determined as described hereinafter in the examples.

Several food products may be prepared according to the invention, for example, spreads, soups, noodles, ice-cream,

saucers, dressing, snacks, cereals, beverages, bread, biscuits, other bakery products, sweets, bars, chocolate, chewing gum, dairy products, dietetic products e.g. slimming products or meal replacers etc.

5

The statins and soy actives are present in the food product in an amount sufficient to obtain a blood LDL-cholesterol lowering effect if the food product is used according to the common needs of the consumer.

10

The skilled person will be able to adjust the percentage of statins and soy actives in the food product to get the above effect. The percentages will depend on the type of food product, since the food products are used in different serving sizes. Moreover the pattern in a food product is consumed (servings per day and distribution over days) is dependent on the food product.

Preferably in the food products according to the invention, the amount of statin is 5-500 mg/kg and the amount of soy actives is 1 wt. or higher. More preferably the the amount of soy actives is 5 wt.% or higher or 10 wt% or higher. Most preferably the amount of soy actives is 20 wt.% or higher.

As an illustration table 2 indicates a number of products, which may be prepared according to the invention, and a typical serving size.

Table 2

Product	Daily Serving
Margarine	15 g
Meat product	50 g
Dressing	30 g
Sweet	10 g
Bar	75 g
Meal replacer drink	330 ml
Beverages	200 ml

Preferably the food product according to the invention is a  
5 spread, meat product, sauce, soup, bakery good, beverage or  
bar. These products are preferred because the way in which they  
are consumed results in a more constant intake of statins and  
soy actives than in other food products. More preferred food  
products according to the invention are a spread, cereal bar,  
10 beverage or breakfast cereal.

The invention will now be further illustrated by the  
description of suitable embodiments of the more preferred food  
products. It belongs to the ability of the skilled person to  
15 use the teaching provided therewith to prepare other products  
of the invention.

- Spreads

20 Typically, spreads according to the invention are oil in water or  
water in oil emulsions, although also spreads, which are  
substantially fat free, are covered. Spreads are to include  
margarines and liquid cooking products. The spreads may be  
spreadable and not pourable or may be pourable at the temperature  
25 of use e.g. 2-10 °C. Fat levels may vary in a wide range e.g.



11

full fat margarines with 60-90 wt.% of fat, medium fat margarines with 30-60 wt.% of fat, low fat products with 10-30 wt.% of fat and very low or fat free margarines with 0 to 10 wt.% of fat.

5 The fat in the margarine or other spread may be any edible fat, often used are soybean oil, rapeseed oil, sunflower oil and palm oil. Fats may be used as such or in modified form e.g. hydrogenated, esterified, refined etc. Other suitable oils are well known in the art and may be selected as desired.

10

The pH of a margarine or spread may advantageously be from 5.0 to 6.5, though other pH's are possible.

Examples of spreads other than margarines are cheese spreads,  
15 sweet spreads, yoghurt spreads etc.

Optional further ingredients of spreads may be emulsifiers, colourants, vitamins, preservatives, emulsifiers, gums, thickeners etc. The balance of the product will normally be  
20 water.

A typical size for an average serving of margarine or other spreads is 15 grams. Preferred levels of statins in the margarine or spread are: 20-500 mg/kg statin, more preferred ranges are 50  
25 to 250 mg/kg statin.

- Beverages

Preferred food products according to the invention are beverages,  
30 for example tea, fruit juice, soft drinks, meal-replacers, etc. Meal replacer drinks will be described in more detail herein below. It will be apparent that similar levels and compositions apply to other beverages comprising statins and soy actives.

A typical serving size of a beverage is taken to be 200 ml.  
Preferred levels of statins in a beverage are: 5-100 mg/kg, more preferably 10-80 mg/kg.

5

- Bars, including cereal bars

These products often comprise a matrix of edible material  
10 wherein the statin and soy actives can be incorporated. For  
example the matrix may be fat based (e.g. couverture or  
chocolate) or may be based on bakery products (bread, dough,  
cookies etc). Preferably the food product is a cereal bar, in  
which the matrix is based on agglomerated cereal particles  
15 (rice, grain, nuts, raisins, fruit particles).

The matrix material of a bar may be present in an amount of 60-  
95wt.% of the weight of the bar, preferably 70-90 wt.% most  
preferred 75-85 wt%.

20

Other ingredients in the cereal bar may be starch, sugar (e.g.  
0-10wt%), sirups, honey, milk solids, salt (e.g. 0-5 wt.%)  
calcium carbonate, vitamins, flavouring and colouring.

25 The ingredients are usually mixed and cooked (e.g. by cooking-  
extruding) to produce the (cereal) bar.

A typical size for a bar could be from 20 to 200 g, generally  
from 40 to 100 g. Preferred level in such products would be: 25  
30 to 500 mg/kg statin. More preferred range for this level is 50 to  
300 mg/kg.

Further ingredients may be added to the product such as flavouring materials, vitamins, minerals etc.

#### Preparation of the food product

5

According to the invention a substrate, prepared from soybeans and/or ingredients thereof is fermented with a *Monascus* fungus and the fermentation product is used in the preparation of a food product. These steps will be illustrated below.

10

Fermentation is conducted in known way. The fermentation is conducted in at least one fermentation vessel (fermenter) in which a medium comprising soybeans and/or ingredients thereof is present. The fermentation is started (inoculated) by adding  
15 a suspension of spores of the *Monascus* fungus (inoculum), which has been prepared by fermenting *Monascus* fungus on a separate medium.

The fermentation involves the following steps, which are  
20 executed in the given order:

- a) Preparation of the medium for the inoculum and the medium to be used in the fermenter
- b) Sterilization of the media, fermenters and ancillary equipment
- 25 c) Production of inoculum
- d) Addition of the inoculum to the medium comprising soybeans and/or ingredients thereof, in the fermenter.
- e) Conducting the fermentation
- f) Removal of the fermentation product from the fermenter

30

The fermentation product is used in the preparation of the food products according to the invention.

Optionally, before the fermentation product is used in the preparation of the food products, the following additional process steps may be executed:

- 5 g) Sterilization of the fermentation product
- h) Drying of the fermentation product (or sterilized fermentation product)
- i) One or more separation steps, for instance extraction, to separate statins and soy actives from *Monascus* biomass in
- 10 the fermentation product

The medium used in the fermenter may be solid or liquid. Advantageously the medium is solid, most preferred the medium substantially consists of crushed whole soybeans, which

15 have been soaked with water (e.g. 30 wt% water). In case the medium is liquid, usually water is present as major constituent of the medium.

Whole soybean are preferably used as a substrate for the

20 fermentation. Typical composition of whole soybeans is 42% wt% protein, 20 wt% oil, 35% wt% total carbohydrates, 5 wt% ash and 5.5 wt% crude fiber (Kawamura, S., Tech. Bull. Faculty Agric., Kagawa Univ., 18, 117 (1967)).

25 Instead of whole soybeans, parts or ingredients of soybean may be used in the medium for the fermenter, for instance soy protein (including textured vegetable protein), soy milk, soy-flakes etc. Care has to be taken that the medium contains compounds that can provide a carbon source and a nitrogen

30 source for growth of the *Monascus* fungus.

15

The *Monascus* fungus used according to the invention may be any *Monascus* fungus that produces statins. Preferably the fungus is chosen from the group of *Monascus ruber*.  
Most preferred is *Monascus ruber* F125 M1-4.

5

Strains F125 and F125 M1-4 are deposited at the Centraal Bureau voor Schimmelculturen (CBS) as no. CBS 109070 on 14.11.2000 and no. CBS 109269 on 23.01.2001.

10 These deposit were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty).

15 Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

20 The medium will ordinarily be sterilized before fermentation, e.g. by heat treatment, like pasteurization.

The medium in the fermenter may contain other substances, which may aid the fermentation, for instance sugars, amino acids and

25 vitamins.

The fermentation may be carried out in a manner, which can be determined by the skilled person on the basis of common general knowledge of fermentation technology. As illustration preferred  
30 embodiments are described hereunder.

The fermentation temperature may be important. The temperature is preferably in the range of 10 to 37, °C more preferably 20 to

30°C. We have found that at 37°C and higher the production of statins decreases.

Preferably during fermentation the medium is aerated, e.g. by stirring, shaking etc. Aeration may be carried out by blowing air through the fermentation medium. Preferably the air is wholly or partly saturated with water vapour in case solid state fermentation is used. This avoids drying out of the fermentation medium.

10

The relative levels of statins to soy actives will depend on the fermentation time. The fermentation time is therefore dependent on the desired amount of statins in the fermentation product. Preferred fermentation time is 1-50 days, more preferably 15-40 days, most preferably 20-30 days.

Prior to addition to the food product, the fermentation product may be subjected to a separation step, to separate statins and soy actives from *Monascus* biomass in the fermentation product. This separation may be done with known separation techniques, e.g. filtration or centrifugation.

The fermentation product may also be extracted and the extract may be used in the preparation of the food product. Preferred extraction agents are food-grade extraction agents. More preferred extraction agent is ethanol. Most preferred extraction agent is vegetable oil, e.g. soybean oil or sunflower oil.

The extraction may be done on the fermentation product. Alternatively the *Monascus* biomass may be separated from the fermentation product prior to extraction, e.g. by filtration. The *Monascus* biomass may be separately extracted and the

resulting extract can also be used in the preparation of the food product.

Extracts may be used as such in the preparation of food products. Preferably extraction solvent may be removed from the extracts, e.g. by evaporation of the extraction solvent.

The fermentation product (including extracts etc.) may directly be added to food product ingredients in the process of preparation of the food products according to the invention. It can be added to the other ingredients of the food product composition, or it may be added to part of the ingredients, before other ingredients are added. If more than one phase is present in the food product the fermentation product may be present in one or more of these phases. Preferably the fermentation product will substantially be present in an oil phase, if such oil phase is present.

The invention will be further illustrated in the examples.

## Examples

### Example 1

#### A. Preparation of *Monascus* strain F125 M1-4

*Monascus ruber* strain F125 was cultivated in malt water liquid medium at 30°C for 4 days. Of this culture, 1 ml was used as an inoculum for a Hybond-N filter (Amersham, UK) placed on a YE plate (4% glucose, 0.3%  $\text{KH}_2\text{PO}_4$ , 1.0% yeast extract (Difco), 1.5% agar). After 3 days incubation at 30°C, the spores were harvested by washing the filters with 10 ml physiological saline containing 0.1% Tween 80. The spores were filtered 4

times through Mira cloth filters to obtain a hyphae free spore suspension. This suspension was used for subsequent mutagenesis.

5 The spores were diluted to a concentration of  $10^8$  spore/ml then exposed to UV light at an intensity of  $100 \text{ joules/m}^2$ . The mutagenised spores were plated on Potato Dextrose Agar (Oxoid) and incubated for 3 weeks. One of the resulting colonies, which had a lighter colour than the others was selected and is herein  
10 defined as *Monascus* strain F125 M1-4.

#### B. Preparation of *Monascus* spores

*Monascus* f125M1-4 spores were prepared by harvesting *Monascus* mycelium from a PDA (potato dextrose agar) (oxoid) slope by  
15 washing with 5 ml physiological saline and incubating the mycelium in 150 ml malt water (oxoid) for 4 days at  $30^\circ\text{C}$ . The spores were harvested by filtration through a Mira cloth filter.

#### 20 C. Fermentation

##### C.1. Preparation of an inoculum

A shake flask containing soybeans is inoculated with filamentous spores suspended in a physiological water solution containing 0.1 wt% Tween 80 (polyoxyethylene sorbitan fatty  
25 acid ester, available from ICI Specialty Chemicals<sup>TM</sup>). This shake flask is incubated to let the mould grow. This results in a *Monascus* spore suspension, which was adjusted by dilution to a concentration of  $1 \times 10^6$  spores/ml.

30

##### C.2. Preparation of fermentation medium

1 kg of soybeans is soaked in tap water ( $50^\circ\text{C}$ ) for 30 minutes. After soaking the beans are rinsed with cold tap water.



Subsequently 50 g batches of the soaked beans (97% soybean) were brought in to 300 ml Erlenmeyer flasks.

### C.3. Fermentation

5 The shake flasks were each inoculated with 1 ml of the prepared *Monascus* spore suspension and incubated for 30 days at 30 °C. A sample from the shake flask was taken every week to monitor the statin production.

10 After fermentation, about 600 g soybeans remained.

### D. Statin analysis of fermentation product

The fermentation product samples are extracted in a 50 ml tube  
15 (Falcon) by adding 6 ml of a mixture of acetonitril, water and phosphoric acid (1:1:0.05, v/v/v)

The mixture is blended with an Ultratorax for 1 min. The mixture was then incubated at room temperature on a rollerbank for over 24 hours. Hereafter the samples were centrifuged and  
20 the supernatant liquid used for HPLC analysis. Samples were separated using HPLC analysis on a Shimadzu apparatus according to the method of Morovjan et al., J. chromatogr. A 763 (1997) 165-172. The system consists of the Shimadzu SCL-10A system controller, CTO-10AS column oven, LC-10AT vp pump system, RID-  
25 10A refraction index detector, SPD-M10A diode array detector and SIL-10AD autoinjector. For the chromatographic determination of statins a Waters NovaPak C18 (150x3.9 mm I.D., 4µm) column was used operating at 25°C. The eluent was acetonitril-0.1% phosphoric acid (50:50,v/v) solution flowing  
30 at 1.5 ml/min. Runs were performed for 15 min. The detection was performed using a diode array detector from 190 nm up to 800 nm. The sum of the area of all peaks in the spectrum belonging to statins is measured. Comparison to a standard

20

(Mevinolin, Sigma) allows the calculation of a statin content (expressed in mg/kg analysed product).

The analysis results in a statin content of 1200 mg/kg.  
5 product.

#### E. Extraction of the fermentation product

The fermentation product was extracted with ethanol and acetonitril. The soybean extracts contained 0.0545 g statin/kg  
10 (ethanol extract) or 0.0978 g statin/kg (acetonitril extract) as determined by HPLC, as described under D.

#### F. Colour analysis of extract

When colours are classified, they can be broken down into the  
15 three primary elements. One is the Hue (colour) the other is Value (brightness) and the third is Chroma (Saturation like vivid colours or dull colours).

To enable anyone to tell anyone else exactly what colour they are talking about a common numerical code is used. This  
20 numerical code used is  $L^*a^*b^*$ . When a colour is expressed in this system, Value becomes  $L^*$ , while Hue and Hue are expressed as  $a^*$  and  $b^*$  respectively. The  $L^*a^*b^*$  was measured of different time samples during fermentation. The supernatant of the samples was filtered sterilized with a milipore 0.22 $\mu$ m filter.  
25 Of the clear liquid  $L^*a^*b^*$  was measured with a UV 1601 spectofotometer of Shimatzu.

$L^*a^*b^*$  values of the soy extracts :

30

Soy fermented with *M. ruber* M1-4

$L^*= 90.2$  ;  $a^*=-4.8$  ;  $b^*=33.8$

21

G. Preparation of a pourable margarine

An ethanol extract of the fermentation product was used for further processing. Ethanol was removed by means of rotary evaporator. The residue was used in a pourable margarine composition. A pourable margarine composition without fermentation product residue was used for comparison with respect to colour.

The composition of the pourable margarine is given in table 3.

10

Table 3. Composition of the pourable margarine of example 1.

Ingredient	Amount (wt.% on total)
Sunflower oil	79.6
Rapeseed oil	1.95
hardened to a melting temperature of 70 °C	
Lecithin (BOLEC-MT)	0.18
Lecithin (Cetinol)	0.2
Potassium sorbate	0.0125
Water	18.0
Statins	0.00011

15

The colour of the pourable margarine was not different from a control with no statins extract, but 20 wt% water.

Comparative example A

Example 1, steps B to F were repeated, but instead of *Monascus* strain F125 M1-4, a *Monascus* strain isolated from red rice (commercial product from China) was used.

The  $L^*a^*b^*$  values of the extracts were determined according to example 1, step F. These values were:

10

$L^* = 90.2$  ;  $a^* = 56.1$ ;  $b^* = 63.9$

Comparative example B

15 The procedure of example 1, steps B to F, was repeated, however in step C.2., instead of 1 kg of soybeans, 1 kg of rice (Parboiled rice, *Oryza*) was used. After fermentation about 500 g rice remained.

20 The  $L^*a^*b^*$  values of the extracts were determined according to example 1, step F. These values were

$L^* = 80$  ;  $a^* = 54$ ;  $b^* = 49$

25

Example 2

A spread having the composition as in table 4 was prepared.

Table 4: Composition of spread of example 2

Ingredient	Amount (wt% on total)
Sunflower oil	69.295
Fat blend	10.5
Beta carotene	0.125
Monoglyceride (Hymono 8903)	0.08
Water	13.33
Ethanol extract of example 1C	6.67
Total	100

The fat blend was a mixture of 65 wt% mid fraction of palm oil 5 stearin and 35 wt% palm kernel oil.

The spread was prepared by premixing the ingredients in a premix tank at 55°C in a premix tank and feeding the premix to a Votator with two scraped surface heat exchangers (A-units) 10 operated at 800 rotations per minute (rpm) and one crystallizer (C-unit) operated at 200 rpm. The configuration was A-A-C.

The amount of statin in the spread was 74 mg/kg. The colour of the spread was not different from a control with no statins 15 extract, but 20 wt.% water.



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## Claims

1. Process for the preparation of one or more statins by fermentation, wherein a substrate is fermented with statins producing fungus, characterized in that substrate comprises more than 20% by weight of soy ingredients.
2. Process according to claim 1, wherein the substrate comprises more than 50% by weight of soy ingredients.
3. Process according to claim 2, wherein the substrate comprises more than 80% by weight of soy ingredients.
4. Process according to any one of claims 1 to 3, wherein the statins producing fungus is a *Monascus* fungus.
5. The *Monascus* strain F125 M1-4 deposited at the Centraal Bureau voor Schimmelculturen on 14.11.2000 having number CBS 109070.
6. Food product comprising:
  - a) an amount of one or more statins
  - b) an amount of one or more compounds chosen from the group: polyunsaturated fatty acids, phytosterols, proteins, peptides, dietary fibers, polyphenols and saponins,wherein the food product has a Hue a\* value less than 20.
7. Food product according to claim 6, wherein the amount of a) is 5-500 mg/kg and the amount of b) 1 wt. or higher.
8. Food product according to claim 7, wherein the amount of b) is 5 wt.% or higher.

25

9. Food product according any one of claims 6-8, wherein the the food product the food product has a Hue a\* value less than 10.

10. Food product according to any one of claims 6-9, wherein the one or more compounds chosen from the group: polyunsaturated fatty acids, phytosterols, proteins, peptides, dietary fibers, polyphenols and saponins are substantially derived from soybeans.

11. Food product according to claim 9 or 10, wherein the food product is a margarine, dressing, sweet, bar meal replacer, breakfast cereal or beverage.



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**Abstract**

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A process is described for the preparation of one or more statins by fermentation, wherein a substrate is fermented with statins producing fungus wherein the substrate comprises more than 20% by weight of soy ingredients. Further a food product is described comprising an amount of one or more statins and an amount of one or more compounds chosen from the group: polyunsaturated fatty acids, phytosterols, proteins, peptides, dietary fibers, polyphenols and saponins, wherein the food product has a Hue  $a^*$  value less than 20.

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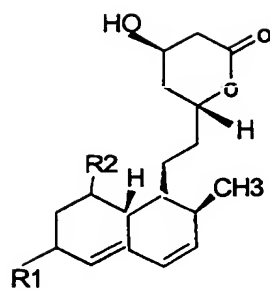
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FIGURE I



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